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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Lui et al. Confirmation No: 1166

Serial No.: 10/540,191 Group Art Unit: 1796

Filed: June 20, 2005 Examiner: A. Khan

For: Method For Bleaching Cellulosic Material With Carbohydrate Oxidases (COX)

DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Yucheng Zhou, do hereby state and declare that

1. I obtained a Bachelor's degree in Chemistry in July 2001 from Beijing Normal University and a Doctor of Philosophy degree in Organic Chemistry in July 2007 from Chinese Academy of Sciences, Institute of Chemistry. From July 2007 to June 2008, I was employed as a Research Scientist by General Electric, China Technology Center, where I worked on the development of new engineering plastics, in particular a polycarbonate product. In July 2008, I joined Procter & Gamble, Beijing Technical Center as a Scientist where my responsibilities included developing a new formulation of laundry powder products. Since July 2009, I have been employed as a Research Scientist by Novozymes (China) Investment Co. Ltd. a subsidiary of Novozymes A/S. At Novozymes, I have worked on developing new enzymes for use in textiles and process development for textile processing, including wet processing, such as desizing, bioscouring, bleach clean-up, biopolishing, denim abrasion, and denim finishing, as well as new textile applications. Novozymes North America, Inc., the assignee of the above-identified application, is also a subsidiary of Novozymes A/S.

2. I have read and am familiar with the present patent application.

3. The following experiments were carried out under my direction and supervision.

4. An experiment was performed to compare the bleaching performance of an *Aspergillus niger* glucose oxidase (GOX) and a *Microdochium nivale* carbohydrate oxidase (COX) on knitted fabric as follows:

A pH 6 solution (adjusted by acetic acid and deionized water) in an amount of 100 ml was added to a 500 ml Launder-O-Meter beaker. Leophen M (a surfactant commercially available from BASF), Emulsifier NA (a surfactant commercially available from Shanghai Shan Chemical Co. Ltd.), Scourzyme L (a *Bacillus licheniformis* pectate lyase product commercially available from Novozymes A/S), Cellusoft CR (EG) (a *Thielavia terrestis* endoglucanase product commercially available from Novozymes A/S), Novoprime A328 (a *Trichoderma reesei* multi-component cellulase product commercially available from Novozymes A/S), and a *Microdochium nivale* carbohydrate oxidase or an *Aspergillus niger* glucose oxidase, were added to the beaker. The gerge knitted fabric (10 g) was added to the beaker, and the fabric was agitated at 42 rpm with 20 steel balls (each steel ball weighed 11.1 g). After the treatment, NaOH was added and the temperature was raised to 95°C for 60 minutes to bleach the fabric. The enzymes were inactivated during this stage. The fabric was rinsed in water at 110°C for 30 minutes before conditioned overnight.

This experiment was performed eight times with different amounts of enzymes, as provided in the following table:

Beaker #	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**
Scourzyme L, g/L		0.4	0.4	0.4	0.4	0.4	0.4	0.4
Cellusoft CR, g/L			1	1	1	1	1	1
Novoprime A 328, g/L				0.8	0.8	0.8	0.8	0.8
GOX, g Enzyme Protein/kg fabric					0.05	0.2		
COX, g Enzyme Protein/kg fabric							0.05	0.2
Leophen M, g/L	1							
Emusifier NA, g/L	1							

The bleaching effect was evaluated by measuring CIE whiteness using a Datacolor apparatus.

The results are provided in the following table:

Beaker #	B1	B2	B3	B4	B5	B6	B7	B8
CIE whiteness	42.77	40.72	41.51	39.93	40.47	40.61	46.11	46.58

The results show that the *Microdochium nivale* carbohydrate oxidase (COX) has a better bleaching performance than the *Aspergillus niger* glucose oxidase (GOX) at the same protein load. In particular, the CIE whiteness using COX was 46.11 and 46.58 and the CIE whiteness using GOX was 40.47 and 40.61. Thus, COX increased the CIE whiteness by approximately 6 units compared with GOX. These results are surprising and unexpected.

5. Another experiment was performed to compare the bleaching performance of an *Aspergillus niger* glucose oxidase (GOX) and a *Microdochium nivale* carbohydrate oxidase (COX) on woven fabric. The woven viisco fabric (10 g) was treated in a 500 ml Labomat beaker containing 100 ml deionized water, Leophen M (a surfactant commercially available from BASF), Emulsifier NA (a surfactant commercially available from Shanghai Shan Chemical Co. Ltd.), Aquazyme® (an alpha-amylase product commercially available from Novozymes A/S), a glucoamylase, and either COX or GOX at 50°C for 60 minutes. After the enzyme treatment, NaOH was added, and the temperature was raised to 95°C for 60 minutes to bleach the fabric. The enzymes were inactivated during this stage. The fabric was rinsed in water (two hot rinses and two cold rinses) and then dried before conditioned overnight.

This experiment was performed four times with different amounts of enzymes, as provided in the following table:

Beaker #	B1	B2	B3	B4
Aquazyme SD-L, g/L	0.8	0.8	0.8	0.8
Glucoamylase, g/L	0.8	0.8	0.8	0.8
GOX, g Enzyme Protein/kg fabric	0.05	0.2		
COX, g Enzyme Protein/kg fabric			0.05	0.2
Leophen M, g/L	0.5			
Emusifier NA, g/L	0.5			

The bleaching effect was evaluated by measuring CIE whiteness using a Datacolor apparatus. The results are provided in the following table:

Beaker #	B1	B2	B3	B4
CIE Whiteness	28.43	31.52	39.77	40.15

These results show that *Microdochium nivale carbohydrate oxidase (COX)* has a better bleaching performance than the *Aspergillus niger* glucose oxidase (GOX) at the same protein load. In particular, the CIE whiteness using COX was 39.77 and 40.15 and the CIE whiteness using GOX was 28.43 and 31.52. Thus, COX increased the CIE whiteness by approximately 8-12 units compared with GOX treatment. These results are surprising and unexpected.

6. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that the statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 26th day
of September 2010

Yucheng Zhou